Vestibulospinal and Reticulospinal Neuronal Activity During Locomotion in the Intact Cat. I. Walking on a Level Surface

KIYOJI MATSUYAMA\(^1\) AND TREVOR DREW\(^2\)
\(^1\)Laboratory of Neurobiology, National Institute for Physiological Sciences, Okazaki 444, Japan; and \(^2\)Department of Physiology, Faculty of Medicine, University of Montreal, Montreal, Quebec H3C 3J7, Canada

Received 7 December 1999; accepted in final form 20 July 2000

Matsuyama, Kiyoji and Trevor Drew. Vestibulospinal and reticulospinal neuronal activity during locomotion in the intact cat. I. Walking on a level surface. J Neurophysiol 84: 2237–2256, 2000. To examine the function of descending brain stem pathways in the control of locomotion, we have characterized the discharge patterns of identified vestibulo- and reticulospinal neurons (VSNs and RSNs, respectively) recorded from the lateral vestibular nucleus (LVN) and the medullary reticular formation (MRF), during treadmill walking. Data during locomotion were obtained for 44 VSNs and for 63 RSNs. The discharge frequency of most VSNs (42/44) was phasically modulated in phase with the locomotor rhythm and the averaged peak discharge frequency ranged from 41 to 165 Hz (mean = 92.8 Hz). We identified three classes of VSNs based on their discharge pattern. Type A, or double peak, VSNs (20/44 neurons, 46%) showed two peaks and two troughs of activity in each step cycle. One of the peaks was time-locked to the activity of extensor muscles in the ipsilateral hindlimb while the other occurred anti-phase to this period of activity. Type B, or single pause, neurons (13/44 neurons, 30%) were characterized by a tonic or irregular discharge that was interrupted by a single pronounced and brief period of decreased activity that occurred just before the onset of swing in the ipsilateral hindlimb; some type B VSNs also exhibited a brief pulse of activity just preceding this decrease. Type C, or single peak, neurons (9/44 neurons, 23%) exhibited a single period of increased activity that, in most cells, was time-locked to the burst of activity of either extensor or flexor muscles of a single limb. The population of RSNs that we recorded included neurons that showed phasic activity related to the activity of flexor or extensor muscles [electromyographically (EMG) related, 26/63, 41%], those that were phasically active but whose activity was not time-locked to the activity of any of the recorded muscles (13/63, 21%) and those that were completely unrelated to locomotion (24/63, 38%). Most of the EMG-related RSNs showed one (15/26) or two (11/26) clear phasic bursts of activity that were temporally related to either flexor or extensor muscles. The discharge pattern of double-burst RSNs covaried with ipsilateral and contralateral flexor muscles. Peak averaged discharge activity in these EMG-related RSNs ranged from 4 to 98 Hz (mean = 35.2 Hz). We discuss the possibility that most VSNs regulate the overall activity of extensor muscles in the four limbs while RSNs provide a more specific signal that has the flexibility to modulate the activity of groups of flexor and extensor muscles, in either a single or in multiple limbs.

INTRODUCTION

The vestibulospinal system provides a large part of the excitatory drive to the antigravity extensor muscles that is necessary for maintaining the normal posture of a terrestrial quadruped or biped both at rest and during locomotion (see Armstrong 1986; Wilson and Peterson 1981 for general references). Indeed it has been known since at least the classical studies of Fulton et al. (1930) and Bach and Magoun (1947) that destruction of the lateral vestibular nucleus (LVN) in the decerebrate cat leads to a significant loss of tone in the extensor muscles. In the intact animal, bilateral labyrinthectomy (Marchand and Amblard 1990; McKinley and Smith 1983; Thomson et al. 1991; Watt 1976), lesion of the vestibular nuclei (Yu and Eidelberg 1981), or lesion of the ventromedial funiculi (Bem et al. 1995; Brustein and Rossignol 1998; Gorska et al. 1990, 1993) all lead to an immediate decrease in extensor muscle tone together with a characteristic series of deficits, including an inability to maintain a stable posture, particularly during movement of the head.

During locomotion, it is presumably the vestibulospinal system, in conjunction with the reticulospinal system (see Mori 1987; Mori et al. 1992), that ensures an appropriate level of muscle tonus in the extensor muscles and that acts to modify that muscle tone in response to changes in the orientation of the head and/or the body. In support of this generalization, results from lesions studies in both the decerebrate (Orlovsky 1972a; although see Jell et al. 1985) and in the otherwise intact animal (Bem et al. 1995; Brustein and Rossignol 1998; Gorska et al. 1990, 1993; Yu and Eidelberg 1981) have shown that bilateral destruction of the LVN, or of the ventromedial spinal cord, results in a loss of extensor tone during locomotion, expressed in the appearance of a crouched gait, as well as in some loss of interlimb coordination. In addition, stimulation of the LVN during the stance phase of locomotion, when most extensor muscles are active, produces an increase in the level of activity in the extensor muscles while stimulation during the swing phase, when most flexor muscles are active, has little effect (Orlovsky 1972a; Russel and Zajac 1979). In several cases, stimulation of Deiters’ nucleus, at least in the fictive preparation, has been reported to be capable of resetting the locomotor rhythm (Degtyarenko 1993; Leblond and Gossard 1997; Russel and Zajac 1979).

Concerning the descending command that is encoded by neurons within Deiters’ nucleus, there is much less information. With the exception of a single brief report (Batson and Armstrong 1980), all studies on the discharge activity of ves-
tibulospinal neurons (VSNs) during locomotion have been performed in the decerebrate cat (Kanaya et al. 1985; Orlovsky 1972b; Orlovsky and Pavlova 1972; Udo et al. 1976, 1982) or guinea pig (Marlinsky 1989, 1992). All of these studies have emphasized that VSNs discharge phasically during locomotion although the description of these discharges varies in the different studies. In both the studies of Orlovsky (1972b) and of Marlinsky (1992), recordings were made while the limbs of only one girdle were active, the hindlimbs in the study of Orlovsky and the forelimbs in that of Marlinsky. In each of these studies, VSNs were described as exhibiting, predominantly, one peak of activity during locomotion, related either to the hindlimb extensors or the forelimb extensors, respectively. In contrast, in the studies of Udo (Udo et al. 1976, 1982), in which all four limbs were walking, VSNs were described as discharging with two clear peaks of activity in each step cycle, with one peak occurring at the end of stance and the other during the swing phase of the ipsilateral limb (see also Kanaya et al. 1985). Moreover, in these studies, it was emphasized that the full pattern of discharge activity was observed only when the cat walked with all four limbs.

Given the lack of information on the discharge patterns of VSNs in the intact animal, we concentrate in this first paper on a characterization of the discharge patterns of VSNs during locomotion and a consideration of what might be responsible for the multiple peaks that were observed in these neurons. The need to characterize VSNs in the intact cat during level locomotion is particularly important as all of the previous studies on VSN discharge patterns were carried out in reduced preparations in which the head was immobilized in a stereotaxic apparatus. Further, given that the other major descending pathway that is involved in the regulation of posture, the reticulospinal system (see Mori 1987; Mori et al. 1992), also receives vestibular input (see Wilson and Peterson 1981), we decided that it was important to directly compare the characteristics of reticulospinal neurons (RSNs), recorded in the same cats, with those of VSNs both during walking on a level surface and on an inclined plane.

A preliminary report of these data has been published in abstract form (Matsuyama and Drew 1996).

M E T H O D S

Animal training and surgery

One female and one male cat (3.2 and 4.8 kg, respectively) were trained over a period of several weeks to walk steadily on a treadmill at a speed of 0.35–0.4 m/s. The cats were trained to walk both with the treadmill in the normal, level orientation and when the treadmill was either pitch tilted (from 20° up to 10° down) or roll tilted (from 20° left side up to 20° right side up).

Following training, the cats were prepared for surgery, which was performed under aseptic conditions. Each cat was anesthetized with pentobarbital sodium (35 mg/kg ip) and administered atropine (40 µg/kg sc). The cats were shaved, washed, and placed in a stereotaxic frame using atraumatic ear bars coated with xylocaine; petroleum jelly was placed over the corneae to prevent desiccation. An intravenous catheter was inserted into the cephalic vein to permit administration of fluids and antibiotics (40,000 IU penicillin G sodium at the beginning and end of surgery).

Using methods that have previously been described (Drew 1993; Drew et al. 1986), each cat was implanted with three 50-µm stainless steel, Tri-ML-insulated, microwire stimulating electrodes (California Fine Wire) into the ventral regions of the spinal cord at L2, and with a recording chamber (internal dimensions, 10×6 mm) that was placed over the cerebellar cortex so as to give access to the LVN and to the pontomedullary reticular formation (PMRF). In addition, pairs of insulated stainless steel wires were inserted into selected fore- and hindlimb muscles to record electromyographic (EMG) activity. In both animals, EMG electrodes were implanted, bilaterally, into the cleidobrachialis (CIB) and the lateral head of the triceps brachii (TriL) in the forelimb and into the anterior head of the sartorius (Srt), the semitendinosus (St), gastrocnemius, lateral head (GL), and vastus lateralis (VL) muscles in the hindlimbs.

Following surgery, the animals were placed in an incubator to recover overnight and were administered an analgesic (Buprenorphine, 5 µg/kg every 6–8 h) for the following 48 h. Experiments began 1 wk following the surgical procedures.

Protocol

In each experiment, a glass-coated, tungsten microelectrode (impedance 0.5–1.5 MΩ) was driven through the cerebellum into the brain stem using a custom-built micromanipulator that was attached to the recording chamber. In each penetration, the most efficient spinal microwire was stimulated (1 pulse of 0.2-ms duration each second) at a strength that preliminary experiments had demonstrated was sufficient to antidromically activate RSNs or VSNs. The tungsten electrode was advanced until an isolated unit was recorded. Each unit was identified as a RSN or a VSN if it responded at a constant latency to stimulation of the spinal cord electrode at 1.5× threshold and, when applicable, if it collided at the appropriate latency with spontaneous action potentials (Lipski 1981). If the unit did not respond at the strength of stimulating current normally used, the stimulus was slowly increased to a predetermined maximum strength (normally that at which distinct stimulus-evoked twitches were observed in the hindlimb musculature). Neurons that did not fire antidromically at these strengths were classified as unidentified and were not tested further.

Identified neurons were recorded during locomotion. In most cases, this recording was carried out in the following order: treadmill pitched up by 20°, then 10°, then level, then 10° pitched down. The treadmill was brought back to level and then rolled 20° left side up; it was then restored to 10° and to level before being rolled 20° right side up and then brought back to 10° and to level. When possible, we then removed the cat from the treadmill to determine the receptive field of the recorded neuron. Unit and EMG data were recorded on a 14-channel instrumental tape recorder. Video recordings (60 fields/s) were taken of some of the experiments using a Panasonic Digital 5100 camera; a digital time code recorded on both the instrumental and the video recorder allowed for synchronization of the two types of data. In tracks in which particularly interesting cells were recorded, small lesions (10–25 µA, DC cathodal current) were made at the area of interest to aid in histological reconstruction.

Data analysis

The present paper addresses only the discharge activity with the cat walking on a level surface while the discharge activity of the neurons on an inclined plane is treated in the companion paper. For the current analyses, step cycles were combined from all of the available sections of level walking that were interspersed between the samples taken during pitch and roll tilt.

Sections of locomotion during which the cat walked regularly in the center of the treadmill belt and in which the amplitude of the single units was stable, were selected for analysis. Single neurons were discriminated on the basis of amplitude and transformed into digital pulses. These digital pulses were sampled at 1 kHz together with the EMGs, which were first filtered at 500 Hz. The onset and offset of each period of EMG activity was marked using an interactive computer program, and each step cycle was allocated an identifying tag to
indicate whether the locomotion was on a level surface or on one of the inclined planes. Data from step cycles recorded in similar circumstances were normalized to 256 bins, and the EMGs were computer-rectified and averaged to form displays of the type shown in Figs. 3–6. For neuronal data, the instantaneous frequency of the discharge (1,000/interspike interval) was calculated prior to normalizing (see Fig. 3A). In all cases, the data were synchronized to the onset of activity in the Srt muscle on the left, ipsilateral (i) side. This muscle was chosen because its period of activity corresponds approximately to the duration of the swing phase of the hindlimb. The time of occurrence of different cell or EMG events is always presented as a phase of the step cycle, where 0.0 indicates the onset of activity in the iSrt and 1.0 indicates the onset of activity in the next burst of activity in sSrt. Neuronal data were also displayed in the form of rasters, in which case cell discharge was synchronized with the onset of activity in a given muscle (see e.g., Figs. 3–6). The temporal relationships between the period of cell discharge and the period of EMG activity in the flexors and extensors of different limbs were used as a guide to determine to which limb the cell was best related and whether it discharged in swing or stance of that limb. Further details of these analyses methods can be found in Drew et al. (1986) and Drew (1993: see also Udo et al. 1982).

Linear regressions were used to determine the relationships between different parameters of the neuronal discharge and of the step cycle; in such cases, the value of the correlation coefficient was used to calculate the probability that the two variables were significantly related. Different characteristics of the populations of neurons and of the EMG activity were examined by using the Student’s t-test (2 tailed) to test the null hypothesis that the two populations were unrelated.

All cells were also examined, off-line, using spike triggered averaging (STA) (see Fetz and Cheney 1980) to determine whether the neuronal discharge produced postspike facilitation or inhibition. For this analysis, we included the neuronal discharges from both level

FIG. 1. Location of the vestibulospinal neurons (VSNs, A) and the reticulospinal neurons (RSNs, B) recorded from the 2 cats used in this study. The location of the neurons has been transposed onto standard sagittal sections of the cat’s brain stem, taken from the atlas of Berman (1968). Note that the neurons in each cat have been collapsed onto a single representative section (laterality indicated at the top right) that best illustrates where the majority of each type of neuron was recorded. 6, abducens nucleus; 7G, genu of the facial nerve; 12, hypoglossal nucleus; CAE, nucleus coeruleus; CX, external cuneate nucleus; DMV, dorsal motor nucleus of the vagus; IOD, dorsal accessory nucleus of the inferior olive; IOMC and IOMR, caudal and rostral divisions of the medial accessory inferior olive; IOP, principal nucleus of the inferior olive; LRI, lateral reticular nucleus, internal division; PH, nucleus praepositus hypoglossi; RM, red nucleus, magnocellular division; TB, trapezoid body; SOL and SOM, lateral and medial nuclei of the superior olive; TRC and TRP central and pericentral divisions of the tegmental reticular nucleus; VIN, inferior vestibular nucleus; VLD, lateral vestibular nucleus, dorsal division; VSN, superior vestibular nucleus.
walking and from locomotion on an inclined plane to ensure that the
largest possible number of action potentials were included in the
analysis. All EMG data were rectified prior to averaging and all action
potentials, regardless of the interspike interval, were included in the
average.

Histology
When recordings had been completed, each cat was deeply anes-
thetized with pentobarbital sodium (40 mg/kg ip), and marking lesions
(35–60 μA, DC cathodal current) were made at predetermined loca-
tions in each recording area. The cats were then perfused transcardi-
ally with 10% formaldehyde, and the brain stem and the lumbar spinal
cord containing the microwires were removed for histological pro-
cessing. The brain stem was sectioned (40 μm) in the sagittal plane
with a cryostat and stained with cresyl violet. The sections were
examined with a microfilm reader and the location of each electrode
track and of the marking lesions traced. Using the marking lesions as
a guide, it was possible to directly identify, or to interpolate, the
location of all of the electrode penetrations that had been made. The
marking lesions were used to identify the location of each of the
recorded cells, and these were then copied onto standard sections
taken from the atlas of Berman (1968) at the appropriate laterality.
This allowed the location of each recorded neuron to be classified
according to its stereotaxic location (see Drew et al. 1986 for further
details).

RESULTS

Unit database

Data were analyzed for a total of 44 VSNs and 63 RSNs
recorded in the two cats. All of the VSNs were histologically
confirmed to lie within the confines of the dorsal division of the
LVN (Fig. 1A: VLD). Although the rostrocaudal and the dor-
soventral extent of the nucleus was explored in both cats, there
was a tendency for the penetrations in cat RS12 to be located
slightly more medially (laterality 3.3–4.1 mm) than those in
RS13 (laterality 3.8–4.6 mm). The RSNs were all recorded
between lateralities of 0.5 and 1.9 mm from the mid-line and
between P6 and P13.4 (Fig. 1B). As such, nearly all of the RSN
recordings were included within the medullary reticular for-
mation (MRF).

Most of the identified neurons that were successfully
recorded during this task were activated at short latency by
stimulation of their axon in the lumbar spinal cord and
therefore had relatively fast conduction times (Fig. 2). For
the VSNs, the mean conduction velocity was 98.5 m/s
(range 63.8–129.6 m/s), while for the RSNs, the mean
conduction velocity of the overall population was 87.1 m/s
(range 34.9–129.6 m/s). The corresponding latency ranges
were from 2.2 to 4.3 ms (mean 2.8 ms) for the VSNs and
from 2.1 to 7.6 ms (mean 3.5 ms) for the RSNs. The
conduction velocities of the subpopulation of RSNs that
were modulated during locomotion (see following text)
were similar to those of the overall population, and ranged
from 34.9 to 121.5 m/s (mean 88.9 m/s).

General discharge characteristics of VSNs during
locomotion

Most VSNs discharged at high frequency and many exhib-
ted a characteristic pattern of two peaks in each step cycle.
Figure 3A illustrates a typical example recorded from one of
the cats (RS13) during level treadmill locomotion. The cell
discharge was characterized by a prolonged burst of activity
that was, in most cycles, clearly composed of two peaks and
of two troughs. This can be more readily appreciated in the
instantaneous frequency record (unit f) illustrated above the
raw unit record of Fig. 3A. These two peaks and troughs are
also clearly seen in the averaged record of cell discharge (unit)
illustrated in Fig. 3B. The most noticeable characteristic of the
pattern of discharge in this neuron was the large decrease in the
discharge frequency (trough 1) that occurred coincidently
with the period of activity in the iSt (Fig. 3, B and C), just prior
to the onset of activity in the hip flexor, Srt. Because the
activity in the flexors and extensors acting around a given joint
are tightly linked, this pause in the discharge pattern also
occurred at the same time as the cessation of the period of
activity of the iVL (Fig. 3C). Linear regressions (Fig. 3D, left)
showed a significant relationship (r = 0.90) between the ter-
mination of the cell discharge and the cessation of activity in
the iVL that was greater than for those obtained with the onset
or offset of any other muscle. Subsequently there was a peak of
activity (peak 1) that occurred during the period of activity of the
iSrt and whose phase of termination (trough 2) was signif-
ically related both to the phase of offset of the iSrt (r = 0.81)
and to the phase of offset of the coGl (r = 0.81, Fig. 3D, right).
Following this diminution, there was a slow and more
prolonged ramp increase in discharge frequency (peak 2) that
paralleled the period of activity of the ipsilateral knee exten-
sor, iVL. Regression coefficients between the phase of onset
and offset of cell discharge in this VSN and the phase of onset and offset of the different muscles that we recorded were greater for the hindlimb muscles than for those of the forelimb. Nevertheless, a regression coefficient of 0.77 was obtained for the relationship between the phase of onset of the coTriL and the onset of peak 1.

An example of a similar discharge pattern recorded from the other cat (RS12) is illustrated in Fig. 4. This VSN also showed two periods of increased discharge frequency in each step cycle (Fig. 4, A and B). As for the example shown in Fig. 3, one period of increased activity occurred during the period of iSrt activity (peak 1) and the other during the period of activity of...
the iVL (peak 2). Again, as for the example in Fig. 3, there was a prominent trough in discharge frequency just prior to the onset of the iSrt (trough 1), but in this case, this cessation of the discharge activity did not align with the iSt but slightly preceded it (Fig. 4C). The second trough (trough 2) was more pronounced than in cat RS13 but occurred at approximately the same time in the step cycle (see Fig. 7A). Although the onset of trough 1 did not align as abruptly with the offset of activity in the iVL as in the previous example, there was, nevertheless, a significant linear relationship ($r = 0.76$) between the beginning of trough 1 and the cessation of activity in the iVL (Fig. 4D) that was greater than with any other of the recorded muscles in either the fore- or hindlimbs. There was equally a significant, linear relationship ($r = 0.84$) between the onset of activity in peak 2 and the offset of activity in the coVL, albeit with a phase delay of $\sim 0.1$. This part of the discharge was equally correlated to the cessation of the activity in the iSrt ($r = 0.87$) and to the onset of activity in the iVL ($r = 0.84$). There were also strong correlations between the onset of activity in peak 1 and the offset of activity in the iSt ($r = 0.81$), between the onset of activity of peak 1 and the onset of activity in the coTril ($r = 0.79$) and between the onset of activity in peak 2 and the onset of activity in the iTriL ($r = 0.84$). The coefficients of correlation with the other muscles were all $\leq 0.6$.

Overall, almost one half of the VSNs recorded in both cat RS13 (13/27; Fig. 5A) and in cat RS12 (7/17; Fig. 6A) showed two such distinct periods of discharge activity and exhibited two troughs, including a clear pause in activity that preceded the onset of activity in the iSrt. We refer to these as type A, or
FIG. 5. Examples of different types of VSNs recorded from cat RS13, organized according to the pattern of discharge. 

A, left: rasters and PEHs, triggered on iSt and iVL, of a type A VSN; right, 4 examples of type A VSNs, including that illustrated to the left (thicker line). The traces are synchronized on the onset of activity in the iSrt and the step cycle is repeated 3 times. The example illustrated with a dotted line is the cell shown in Fig. 3. The 4 cells were recorded in 4 different electrode penetrations. 

B: similar display for 4 examples of type B VSNs. 

C: example of a type C VSN that discharged with a single period of activity. The horizontal line through the PEHs in the rasters synchronized on the iSt illustrates the mean discharge frequency of each of the 3 examples during locomotion. 

D: averaged activity of iSt and iVL (n = 94) taken from the example in C and displayed on the same timebase as for the rasters. 

E: averaged activity (n = 67) of iSrt, iSt and iVL, displayed on the same timebase as for the other averages, and taken from the example illustrated in Fig. 3.
double peak, cells. We defined these cells, objectively, as those VSNs whose peak and trough activity oscillated twice around the mean discharge over the whole step cycle. In most of these cells the initial trough occurred prior to the onset of activity in the iSrt (mean = −0.08) while the second trough occurred subsequent to the end of the iSrt activity at a mean phase of 0.36 (Fig. 7A). The phases of the two peaks in these type A cells were anti-phase, occurring at mean phase values of 0.14 and 0.67 with respect to the onset of activity in the iSrt (Fig. 7B). The durations of the two peaks were, therefore not symmetrical but, as illustrated in Fig. 7C, the duration of the initial peak was significantly shorter ($P < 0.001$) than that of the second.

Although there were no statistical differences in the phase of occurrence of either the peaks or the troughs between cats RS12 and RS13, the discharge patterns in these double burst cells was not identical in the two cats. As can be appreciated from the raster plots of Figs. 3–6, while in cat RS13 trough 1 invariably covaried closely in time with the period of activity in the iSrt, in most, although not all, of the cells recorded in cat RS12, it preceded the period of activity in iSrt. This suggests that the cell discharge may be related to an event that is phase-locked to the onset of activity in iSrt rather than the period of activity in that muscle, or similarly active synergists, per se. In addition, inspection of Figs. 5A and 6A illustrates that

![Diagram of cell discharge patterns](image)

**FIG. 6.** Examples of other VSNs recorded from cat RS12. Data are displayed as in Fig. 5. The dotted line in the averages in A corresponds to the example illustrated in Fig. 4.
some cells showed a ramp increase in activity during peak 2 (as in the cells illustrated in Figs. 3 and 4) while others, especially in cat RS13, exhibited a more discrete phasic increase toward the end of the ipsilateral stance phase (see e.g., Figs. 5A and 10). Nevertheless, in all of these cells, in both cats, but especially in cat RS13, there was a clear temporal covariation between the cessation of activity in the iVL and the pause in cell discharge at the end of peak 2.

The other major pattern of discharge activity that was observed in these VSNs is illustrated in Figs. 5B and 6B. In 8/27 VSNs in cat RS13 and in 5/17 VSNs in cat RS12, the cell discharge was characterized primarily by a single, brief, pause in the discharge frequency during the step cycle. In 12/13 of these cells in both cats, this pause occurred coincidently with or just before the onset of activity in the iSt (in 1 cell the pause occurred during the period of activity of the coSt). As can be seen in Fig. 7D, this pause in activity was normally brief (<20% of the step cycle in 11/12 of these VSNs) and was, on average, shorter than the duration of trough 1 in the double peak, type A cells. During the remainder of the step cycle, the cells showed a mostly tonic or irregular pattern of activity that either stayed above or below the mean frequency for most of the cycle or that crossed the mean frequency several times for short periods. We refer to the cells with these characteristics as type B, or single pause, cells. In cat RS13, this pause was normally preceded by a brief pulse of increased discharge (Fig. 5B). In all of these cells, there was a close correspondence between the offset of activity in iVL and the cessation of cell discharge. In cat RS13, the pulse of increased discharge at the end of the period of activity of the iVL also occurred coincidentally with the onset of activity in coGL; i.e., the maximum activity occurred at the overlap between the two extensors of the two hindlimbs. (Note, however, that as the coGL discharge starts prior to foot contact, this does not correspond to the period of double support; the contralateral hindlimb is, instead, in the E1 phase of extension at the end of swing at this time: see following text.)

Most of the remaining VSNs, 6/27 in RS13 and 3/17 in RS12, exhibited a single peak of discharge activity that oscillated once around the mean frequency of the discharge. These cells are referred to as type C, or single burst, cells. Objectively, these cells were distinguished from the type B cells both by the clear single oscillation around the mean discharge activity and by the longer duration of the trough (Fig. 7D). In most of these cells, the period of phasic activity was time-locked to the period of EMG activity in a single limb. For example, the single period of more intense activity of the VSN illustrated in Fig. 5C covaried with the period of activity of the iVL. Two other VSNs recorded in this same penetration showed a similar pattern of activity. The other three single-peak cells that were recorded in this cat showed more discrete patterns of activity that covaried in time with the period of activity of iSt (1 cell) or coSt (2 cells). In RS12, the activity of two VSNs, recorded in the same penetration, covaried with the period of activity in the coTrIL.

There was no evidence that any one type of VSN was preferentially located in any one part of the LVN and in most penetrations cells of different types were recorded in a single penetration.

**Frequency of discharge activity**

As illustrated in Fig. 8, A and B, averaged discharge rates during the peaks of the activity in type A and B VSNs often
exceeded 100 Hz. Overall, the averaged maximum frequencies observed in either peak in both cats ranged from 41 to 165 Hz (mean = 92.8 Hz). For the type A VSNs, average frequencies during peak 1 were 103 Hz, and during peak 2, they were 98 Hz. In type B cells, average frequencies were, by definition, only available for peak 2 and were taken as the maximum value just preceding the pause. For the 13 type B VSNs illustrated in Fig. 8B, the average discharge frequency was 92 Hz. There

FIG. 9. Averaged activity of a type A VSN from cat RS12 (A) and of a type C VSN from cat RS13 (C), together with duty graphs illustrating the average time of paw contact and paw lift during sections of locomotion including those from which the averaged cell discharge activity was calculated. In the duty graphs, the horizontal bars represent stance (paw contact to paw lift) and the spaces between them indicate swing (paw lift to paw contact). Each step cycle is repeated twice in this figure. The data for both the cell discharge and the duty graphs are synchronized with respect to the onset of the iSrt. The rasters and PEHs of the cell discharge illustrated below the duty graphs (B and D) are triggered on the moment of paw lift (↑) of each of the four limbs (initial, vertical line). The subsequent, staggered, vertical line indicates paw contact (↓) and the second staggered vertical line, when present, indicates paw lift in the next step cycle. The data are rank-ordered with the longest swing phase at the top. LFL, left forelimb; LHL, left hindlimb; RFL, right forelimb; RHL, right hindlimb.
were no significant differences between the peak values in these two groups of cells. Similarly, there were no significant differences in the average discharge frequencies during trough 1 for the type A and B cells (40 and 43 Hz, respectively: Fig. 8D). The average discharge frequency for the type A VSNs during trough 2 was 58 Hz (Fig. 8C).

**Relationship to paw contact and lift**

Given the variability in the discharge patterns with respect to the onset of the iSrt and the time of occurrence of the iSt in cats RS12 and RS13, we investigated whether the time of the peaks and troughs might reflect better the time of paw contact and lift of the different limbs than the activity of the EMGs. We, therefore, measured the time of paw contact and lift in the four limbs for a few cells from each cat in which high-quality video records showing all four limbs were obtained and synchronized with the EMG and cell data using the digital time code that was recorded on the video and instrumental recorders (see Methods). Figure 9 illustrates the results of this analysis for one cell from each of the two cats, representing a type A (Fig. 9, A and B) and a type C (Fig. 9, C and D) discharge pattern. Inspection of the discharge pattern of the cell illustrated in Fig. 9B (bottom left) shows that there was a close temporal covariation between the onset of discharge of peak 2 (P2) and the onset of stance in the LHL (↓). The pause in cell discharge (trough 2) began a fixed time before LHL paw contact (r = 0.93), and cell discharge recommenced (peak 2) at exactly the same time as paw contact (r = 0.95). There was a similar decrease in discharge (trough 1) just before the contact of the RHL, although neither this trough (r = 0.62) nor the subsequent period of activity (peak 1, r = 0.82) was as well correlated with the RHL as peak 2 and trough 2 were with the LHL. The onset of peak 1 also showed a good relationship (r = 0.82) with the time of foot contact of the RFL.

In the cell illustrated in Fig. 9, C and D, there was a clear decrease in the discharge frequency of the cell at the moment of paw lift (↑) of the LHL and a clear increase in activity at the onset of stance of the LHL (Fig. 9D, bottom left). There was no fixed temporal relationship between the cell discharge and the paw lift or contact in any of the other three limbs. Linear regressions between the onset and offset of unit activity and the onset and offset of paw lift and contact of each of the four limbs or of the EMG activity showed a significant relationship of >0.7 only with the onset and offset of the burst of the iSt (not illustrated). The relatively low value for the regression coefficient, compared with those obtained in the other illustrated cells, is most likely because of the difficulty in accurately determining the onset and offset of the individual bursts of unit activity in this cell. Indeed, quantitative analysis on a step-by-step basis was impossible in most VSNs for this same reason.

As stated in the preceding paragraphs, some of the type A VSNs in cat RS13 showed a more phasic pattern of discharge that was time-locked to several events in the step cycle. In the example illustrated in Fig. 10, for example, the onset of the first peak of activity (P1) was well correlated with paw lift of the LHL (r = 0.86), paw contact of the RFL (r = 0.93), and the onset of activity in iSt (r = 0.82, not illustrated). Onset of activity in peak 2 (P2) was less well correlated than that of the first peak (primarily because of the difficulty of accurate detection of this event), but relatively high correlation coefficients were obtained with paw contact of the LFL (r = 0.58), the onset of activity of coSrt (r = 0.68), the offset of activity in the iClB (r = 0.67) and the onset of activity in iTriL (r = 0.70). Indeed, inspection of the step-by-step discharge activity showed that a prolongation of the activity in the iClB always led to a prolongation of the duration of peak 1. Thus the discharge activity in this cell seemed as well related to some of the events in the forelimb as to those in the hindlimbs.

The fact that the discharge activity of some of these neurons increased at the onset of stance of the left and right forelimbs led us to consider, post hoc, that some of these peaks of discharge might be related to the upward acceleration of the body that must be produced by the paw contacts of the limbs.
in a similar manner to the rhythmical vertical displacement of the head that is observed in walking humans (Murray 1967). We, therefore attached small accelerometers (Wilcoxon Research, Model 137) to the head of another cat (implanted for different, unpublished, experiments) to determine how the vertical acceleration acting through the head was modulated during locomotion. As indicated in Fig. 11A, there were two clear peaks of negative acceleration in each step cycle (Accv1 and 2), occurring just after the onset of EMG activity in the forelimb extensors and paw contact of each of the forelimbs. These negative peaks of acceleration correspond to the upward displacement of the head. An analyses of the timing of the peaks of Accv with respect to paw contact and TriL onset is shown in the linear regressions of Fig. 11B. Inspection of these plots shows strong significant linearity between each of these measures, with peak negative acceleration occurring just after paw contact. Linear regressions with the onset and offset of activity in the hindlimb extensors revealed substantially lower regression coefficients than those obtained with the forelimb extensors.

Although these data were taken from a different cat, comparison of the time of occurrence of the negative peaks of acceleration with the times of occurrence of the peaks of cell discharge in Fig. 10 shows an excellent concurrence between the two.

Receptive fields

As these VSNs were recorded for long periods of time while the treadmill orientation was being changed, we were rarely able to maintain cell stability sufficiently long to routinely enable us to remove the cat from the treadmill and to test the peripheral receptive field of the cells. However, we were able to partially or fully test the peripheral receptive fields of 19 VSNs. In 17/19 of these cells, the neurons could be discharged by passive manipulation of one or more limbs. All 17 of these neurons discharged to passive movement of the ipsilateral hindlimb and in 5/14 cells, also to manipulation of the contralateral hindlimb. Fourteen VSNs (14/17) also discharged to manipulation of the ipsilateral forelimb and, when tested, to manipulation of the right forelimb. Finally, 13/17 VSNs clearly

FIG. 11. A: Averaged EMGs and vertical acceleration trace together with a duty graph indicating paw contact and lift. All data are aligned on the onset of iSrt and are taken from cat RS18, from which data have been published only in abstract form (Prentice and Drew 1995). Two cycles are shown, delimited by the heavy, vertical dashed line. The other 2, vertical dotted lines illustrate the relationship between the 2 negative peaks of linear vertical acceleration (Accv1 and Accv2) that are observed in each step cycle and the onset of activity in the iTriL and the coTriL. B: linear regressions between the time of paw contact of the forelimbs, the time of onset of activity in the forelimb extensor, TriL, and the negative peak of activity in the vertical accelerometers. CIB, cleidobrachialis; g, acceleration due to gravity (9.81m/s).
discharged to movement of the head in space, especially when the head of the cat was moved in the horizontal plane, toward the right, contralateral side. Given the relatively small number of VSNs for which a receptive field was verified, we have not tried to establish relationships between the receptive fields and the cell discharge patterns.

**Discharge frequency at rest**

The resting discharge frequency, during quiet standing, of these VSNs was only recorded after the locomotion of the cells had been recorded in all other situations. Consequently, such measures were obtained for only 19 VSNs in the two cats. In general, the mean instantaneous frequency at rest fell between the maximum and minimum values that were observed during locomotion. In cat RS12, the mean frequency, from six cells was 47.3 ± 11.5 (SD) Hz, while in cat RS13, from 13 cells, it was 39.7 ± 14.5 Hz. Comparing these mean values to the mean values for the peaks and troughs for the VSNs (see Fig. 8 and text), it can be seen that, in many cells, both the peaks and troughs during locomotion represent increased discharge compared with quiet standing. This is particularly evident for the two peaks and for trough 2, while the value for trough 1 is closer to the resting value. A similar impression was obtained when the values obtained during quiet standing for individual cells were compared with the discharge of those same cells during locomotion, at least with respect to the maximal discharge (see e.g., the horizontal dotted lines in Figs. 3B and 4B). Thus the discharge frequency of peak 2 in all nine of the type A cells for which data were available was higher than during quiet standing as was the discharge frequency of peak 1 in 7/9 VSNs. However, in 5/9 VSNs the cell also discharged more intensely during trough 1 than it did at rest, and for 7/9 VSNs the same was true for trough 2. Thus the overall discharge frequency in some type A VSNs during locomotion was higher throughout the step cycle than during quiet standing. In all five type B VSNs and all three type C VSNs for which similar data were available the discharge frequency was modulated both above and below the resting discharge frequency.

**Discharge characteristics of RSNs**

One of the major goals of this series of studies was to directly compare the discharge activity of VSNs and RSNs in the same cats under the same conditions. While the companion paper provides new data on the discharge characteristics of RSNs during locomotion on an inclined plane, the present paper provides the base data that are required for comparison between cell populations and between tasks. In general, the population of RSNs recorded in the present study discharged in a similar manner to that described in our earlier publication (Drew et al. 1986). The discharge activity of 26/63 (41%) of the RSNs was phasically modulated, and the onset and/or offset of the bursts of activity covaried with the time of onset and offset of one or more of the limb EMGs we recorded. These cells are referred to as EMG related, and the number of cells related to flexor and extensor muscles in each limb is shown in Table 1. A further 13/63 (21%) also showed phasic modulation of their discharge frequency in each step cycle.

However, this discharge did not covary in any fixed manner with any of the limb EMGs recorded in this study, and, as in our previous publication, we will refer to these as locomotor related. Finally, 24/63 (38%) of the RSNs were either silent during locomotion, discharged irregularly or were completely tonic throughout the step cycle.

Figure 12 shows three examples of EMG-related RSNs recorded from the two cats. As we have previously reported (Drew et al. 1986), a large number of those neurons whose period of discharge activity covaried with that of flexor muscles discharged twice in each step cycle, in a similar manner to the two examples illustrated in Fig. 12, A and B (see also Table 1). In these examples, there was one burst of discharge that overlapped the period of activity of the iSrt, and another burst that overlapped the period of activity of the coSrt. In contrast, most of the extensor-related RSNs discharged in a single burst of activity. Figure 12C illustrates one example of a RSN whose discharge frequency covaried with the period of activity of the iVL.

Reflecting the more varied patterns of discharge that were observed in the RSNs, the phase at which the minimum or maximum discharge occurred was also more variable in these neurons than in the VSNs (see Fig. 13A). For example, the phase of peak discharge in cells with a minimum early in the step cycle (see e.g., Fig. 12C) was frequently later than for peak 1 of the VSNs. In addition, trough 2 for cells that discharged in a double burst (e.g., Fig. 12, A and B) occurred, on average, slightly earlier in the step cycle (phase = 0.31) than it did for the population of VSNs. More noticeable was that the average peak discharge frequencies of the RSNs was considerably lower than for the population of VSNs (Fig. 13B).

While the averaged discharge frequency of 15/20 type A VSNs exceeded 80 Hz in both peaks and 8/13 type B VSNs also showed maximal discharge frequencies of >80 Hz, only 2/39 of the phasically modulated RSNs showed averaged discharge frequencies in this range.

**Spike-triggered averaging**

Spike-triggered averaging was performed on all VSNs that were recorded and on all RSNs that discharged during locomotion using all action potentials in all conditions. In no case was there any evidence of significant post spike facilitation in any of the recorded muscles despite the fact that the mean

<table>
<thead>
<tr>
<th>Table 1. Modulation patterns of EMG-related RSNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexors</td>
</tr>
<tr>
<td>iHL</td>
</tr>
<tr>
<td>coHL</td>
</tr>
<tr>
<td>iFL</td>
</tr>
<tr>
<td>coFL</td>
</tr>
<tr>
<td>Extensors</td>
</tr>
<tr>
<td>iHL</td>
</tr>
<tr>
<td>coHL</td>
</tr>
<tr>
<td>iFL</td>
</tr>
<tr>
<td>coFL</td>
</tr>
</tbody>
</table>

The table shows the number of EMG-related neurons that covaried with flexor or extensor muscles of each of the four limbs. The double-burst cells were related to either the iHL and coHL or to the iFL and coFL. i, ipsilateral; co, contralateral; FL, forelimb; HL, hindlimb; RSN, reticulospinal neuron.
The results in this paper provide the first detailed information of the discharge characteristics of identified vestibulospinal neurons during locomotion in the intact cat. We show that most VSNs discharge intensely throughout the step cycle and exhibit one, or more commonly, two periods of modulated discharge that occur at relatively fixed periods during the step cycle. A direct comparison with the activity of identified reticulospinal neurons shows several differences in the discharge characteristics of these two populations of brain stem neurons, particularly with respect to the pattern and the intensity of the discharge patterns.

**Database**

The data presented in this and the companion paper were obtained from two chronically implanted cats. In the LVN we recorded 44 VSNs, 17 from cat RS12 and 27 from cat RS13 (Fig. 1A). These neurons were all recorded within the dorsal regions of the LVN as would be accepted on the basis of the commonly accepted view of the functional organization of the LVN (Pompeiano and Brodal 1957; Wilson and Peterson 1981). Although VSNs in cat RS12 were recorded in slightly more medial parts of the LVN than in cat RS13, the general characteristics of the cells were similar in each cat. This suggests that our database gives a representative picture of the discharge characteristics of VSNs projecting to the lumbar spinal cord, although more detailed sampling might reveal other, less common, patterns of activity. We would, moreover, also emphasize that all of these VSNs were identified as projecting to the lumbar spinal cord and neurons projecting...
only to the cervical cord or those with no spinal projections might well show different patterns of activity.

Discharge characteristics from RSNs were sampled from 63 neurons, 14 in cat RS12 and 49 in cat RS13. Most of these neurons were found in the MRF, with the majority of this sample being obtained from the nucleus reticularis gigantocellularis. As indicated in RESULTS, the discharge characteristics of this population of neurons were very similar to those described by us previously (Drew et al. 1986).

Although recording identified neurons from both structures in the same cat obviously reduced the number of neurons recorded from either structure, we feel that any uncertainty in the validity of the conclusions on this basis is outweighed by the possibility of directly comparing neuronal characteristics from the two structures most involved in postural regulation in the same cat. This is particularly true for the data in the companion paper in which neuronal activity during locomotion on an inclined plane is presented.

Although we do not believe that there is a spatial bias in our sampling, it is probable that our sample is biased toward large cells with fast conducting axons. Such a bias is to be expected for recordings made in the awake, unrestrained animal and is obvious from the skew of the plots of the conduction velocities shown in Fig. 2. Nevertheless, cells with both fast and slowly conducting axons were recorded and the range and mean of the recorded cells agreed well with those reported previously for both the LVN (Ito et al. 1964; Orlovsky 1972b; Udo et al. 1976; Wilson et al. 1966) and the PMRF (Drew et al. 1986; Ito et al. 1970; Orlovsky 1970; Peterson et al. 1979).

We divided our population of VSNs into three groups based on the profiles of the average discharge activity of the cells and the relationship of that profile to the mean discharge frequency of the VSNs. This simple classification encompassed all except two of the recorded VSNs. As illustrated in Figs. 5–7, neurons contained within a single group all showed characteristics in common that were different from those of VSNs in the other groups. For example, only the type A neurons showed two clear peaks that oscillated around the mean frequency and only the type C neurons showed a single peak of activity that oscillated around the mean frequency. These two groups of neurons were, therefore easily distinguished. The type B neurons were more difficult to classify as their identification relies more on the absence of any clear modulation, except for the pause. However, as illustrated in Figs. 5 and 6, their discharge activity was subjectively, as well as objectively, in most cases quite distinct from the other two classes of VSNs. At the same time, it should be emphasized that these three classifications are quite broad, and it is clear that not all VSNs within a single group discharge in the same manner. Rather as is clear from inspections of Figs. 3–6, 9, and 10, individual neurons, within a single category, could show different patterns of activity and may well have different functions to play. Nevertheless, we believe that the broad classification that we used provides a useful framework in which to discuss the possible functions of different types of VSN.

**Discharge characteristics of type A VSNs**

The most abundant class of neuron that we encountered was the type A VSNs that discharged with two peaks and troughs of activity in each step cycle. Neurons with these characteristics were recorded from both cats (Figs. 3–6) where they made up 41% of the population in cat RS12 and 48% in cat RS13.

The homogeneity in discharge characteristics of this group is evident in the strong temporal correlation between the discharge of the cells and the period of activity of the iVL. Although not all cells showed a ramp increase in activity throughout the period of activity of the iVL (see e.g., Fig. 10), they all showed a strong relationship between the phase in the step cycle at which the iVL became inactive and the phase at which cell discharge stopped or decreased (see Figs. 3–6).

Such a temporal relationship between the discharge activity of VSNs projecting to the lumbar spinal cord and ipsilateral...
hindlimb extensor muscles is to be expected. In the experiments of Orlovsky (1972b), for example, 78% of the modulated VSNs (our recalibration from Orlovsky’s Fig. 3), identified as projecting to the lumbar spinal cord, discharged with a single burst of activity that began at the end of the swing phase and continued into the stance phase. In addition, Orlovsky (1972a) also reported that stimulation of the LVN during stance resulted in an increase in the activity of the ipsilateral hindlimb extensor muscles and that destruction of this nucleus resulted in a diminution of ipsilateral extensor muscle activity (see also Yu and Eidelberg 1981). As these results can be readily explained on the basis of the well-known facilitatory effect of vestibulospinal axons on ipsilateral extensor motoneurons (Grillner et al. 1971; Wilson and Peterson 1981; Wilson and Yoshida 1969), it is logical to conclude that VSNs facilitate ipsilateral extensor muscle activity during locomotion and that this is the role of the increased discharge activity that forms peak 2 in these type A cells. Nevertheless, given the fact that the onset of activity of iVL clearly preceded the onset of cell discharge in many cases (see Figs. 3–6), the VSN discharge during peak 2 is unlikely to be implicitly signaling the overall period of hindlimb extensor muscle activity.

In the experiments of Orlovsky (1972b), most modulated VSNs discharged once in each step cycle while in our experiments all of the type A cells, by our definition, discharged twice. The function of the other period of increased activity (peak 1) is less clear. The fact that both hindlimbs (but neither forelimb) were walking in the experiments of Orlovsky and that most cells discharged in a single burst of activity would suggest that the second peak of activity in these VSNs might be determined more by activity in the forelimbs than in the hindlimbs. This suggestion is also supported by the results of Udo et al. (1976) who showed that VSNs recorded in their decerebrate walking preparations discharged in a double burst only when all four limbs were walking with the increased activity during one peak coinciding with the hindlimb stance and the other with the hindlimb swing. A similar finding was made by Kanaya et al. (1985), also in decerebrate cats walking quadrupedally, although in their experiments the two peaks were most evident only when the cats were walking at a higher speed.

Comparison of our type A discharge patterns with the patterns described by Udo et al. (1976, 1982) shows an excellent correspondence between the data recorded in the decerebrate and the intact cat. In both preparations, VSNs discharged with two clear peaks, and thus two clear troughs, and in both cases, one of these peaks occurred during the period of activity of the ipsilateral hindlimb extensors (our peak 2 and peak B of Udo et al. 1976), and the other appeared coincidentally with the period of ipsilateral hindlimb flexor muscle activity (our peak 1 and peak A of Udo et al. 1976). Stopping locomotor activity in either the forelimbs or the hindlimbs in the four VSNs that were tested in Udo’s experiments markedly altered the pattern of modulation observed in these cells, leading to the suggestion that coordinated movements of all four limbs were necessary to produce the observed pattern of locomotor activity. Such a finding is perhaps not totally unexpected given that a large proportion of the VSNs identified from the lumbar spinal cord branch profusely (Kuze et al. 1999) and may also send a collateral branch to the cervical gray matter (Abzug et al. 1974). In addition, many of those VSNs for which we could identify a receptive field in the present study were activated by passive movements of several of the limbs and experiments in anesthetized cats have shown that there is convergent afferent input in VSNs from both forelimb and hindlimb afferents (Allen et al. 1972; Anderson and Oscarsson 1978a; Wilson et al. 1967). Moreover, as detailed for the cell illustrated in Fig. 10, some of the VSNs that we recorded showed correlations with events in the forelimbs that were as strong as, and occasionally stronger than, those with events in the hindlimbs.

Although these considerations lead to the suggestion that the neuronal activity is reflecting activity in both the fore- and hindlimbs, it is unlikely that this relationship is a simple one. While the most parsimonious interpretation of these data, on the basis of the anatomy, would be that the VSNs regulate the activity of the ipsilateral fore- and hindlimb extensors, inspection of the temporal relationships of the EMG patterns of these muscles (e.g., Figs. 3 and 4) shows that the activation period of peak 1 is quite distinct from that of the iTriL, which is only slightly phase delayed with respect to the iVL. It has to be considered therefore that the activity in peak 1 might reflect better activity in the contralateral limbs. Although the vestibulospinal tract is normally considered as influencing only ipsilateral motoneurons, there is evidence that stimulation of the LVN also results in activation of contralateral extensor muscles, albeit at a longer latency and with a lower amplitude than on the ipsilateral side (Hongo et al. 1975). Such an action may occur via activation of commisural interneurons (Hongo et al. 1975; Kuze et al. 1999; Sugiuichi et al. 1992), as it does with some reticulospinal axons (Matsuyama and Mori 1998; Matsuyama et al. 1997, 1999). Certainly, in our experiments, many of the cells had bilateral receptive fields, and there is other evidence suggesting that a substantial population of VSNs might receive bilateral input from the fore- and hindlimbs (Andersson and Oscarsson 1978a,b; Wilson et al. 1966, 1967). Such a suggestion would also be in agreement with the fact that some of the cells showed a temporal relationship between the end of the coVL (or coGL) and the end of the period of activity in peak 1 (Figs. 3 and 4), similar to the relationship between the end of the iVL and the end of peak 2. It would also agree with the fact that the activity in some cells increased at the moment of paw contact of the RFL (Figs. 9 and 10). However, we did not observe a fixed relationship between the overall period of activity of the contralateral fore- or hindlimb extensors.

This leads to the suggestion that the discharge patterns observed in these VSNs might depend as much on the overall pattern of interlimb coordination between the limbs as on the pattern of activity in any one or pair of limbs. Such a dependency of the discharge activity on the specific relationship of the coupling between activity in different limbs may explain why the discharge activity of the VSNs, on a step-by-step basis was variable. In Fig. 3, for example, while the double-peak characteristics of the neuron are quite clear from the average and from many of the individual step cycles, in other cycles the double peak activity is much less obvious. This dependency may also explain why the characteristics of the cells in RS12 and RS13, despite their close similarity, also showed some consistent differences e.g., the exact time of trough 1 with respect to the onset of activity in iSrt.
Source of the modulation

The discharge characteristics of these cells during locomotion, taken together with the data obtained during quiet standing, suggest that both excitatory and inhibitory inputs are involved in shaping the discharge pattern.

Excitatory input is likely to come from a number of sources, including peripheral input from the limbs (Brodal and Angaut 1967; Pompeiano and Brodal 1957; Wilson et al. 1966, 1967), from the neck (Boyle and Pompeiano 1980, 1981; Brink et al. 1980) and from the labyrinth (Ito et al. 1969; Shinoda et al. 1994; Walberg et al. 1958; Wilson et al. 1967). In the case of the labyrinth, much of the input is likely to be indirect as stimulation of the vestibular nerve evokes few monosynaptic responses in lumbar-projecting VSNs (Wilson et al. 1967). Given that LVN neurons receive afferent input from otolithic receptors (Peterson 1970; Wilson et al. 1978), it is possible that the contact of each forepaw with the treadmill belt, which produces a strong vertical acceleration of the head (Fig. 11), would produce an afferent input to the VSNs that would contribute to the phasic modulation patterns that we observed. Such a suggestion would be supported by the fact that peak activity in some of the double-peak cells (see Fig. 10) occurred at the corresponding time in the step cycle. However, as VSNs and head accelerations were not recorded in the same animals, and as the exact pattern of discharge activity might depend critically on interlimb coordination (see preceding text), we cannot comment further. Nevertheless, such a suggestion would be in agreement with the results of Melville-Jones et al. (1973) showing that the movement of the head produced by hopping results in a sinusoidal modulation of the vertical acceleration (see their Fig. 4 and our Fig. 11). Moreover, these same authors demonstrated that an imposed vertical sinusoidal acceleration of the cat’s head, in the same range (0.12 g) as that observed in the present experiments (0.13 g), produced clear phasic modulation of some vestibular neurons. These authors concluded that “it would be surprising if the resulting vestibulo-spinal reflexes” (produced by the vertical accelerative signals) “did not contribute in a meaningful way to cyclical events in the synthesis of locomotor control.” On the other hand, there is some evidence to suggest that the transmission of vestibular signals to lumbar-projecting VSNs may be attenuated during locomotion (Orlovsky and Pavlova 1972). However, this conclusion needs to be verified for vestibular input in general. The fact that there was no modulation of the activity of the VSNs in the decerebellate cat might further suggest that the pattern of modulation is entirely due to inhibitory sculpting. However, as Orlovsky states, the high discharge rates that were observed in the decerebellate cat were often accompanied by considerable rigidity so that in a cat with normal tonus, it is probable that both excitatory and inhibitory inputs play a role. Increased discharge in VSNs was also observed by Udo et al. (1976) following cerebellar cooling and was also accompanied by increased levels of extensor muscle EMG. The few sources of recordings of simple spike activity from cerebellar vermal Purkinje cells suggests that they also discharge twice in each step cycle (Udo et al. 1981). Although the majority of the data in the experiments of Udo were obtained from cells receiving input only from forelimb nerves and suggested to project to Deiters’ neurons terminating at the cervical level, similar results were also obtained in the few cells suggested to be hindlimb related. This would again agree with a view that the inhibitory input from the Purkinje cells acts to sculpt the output of the VSNs.

Particularly interesting in this respect are the results of Andersson and Oscarsson (1978a,b; see also Allen et al. 1972), who demonstrated a high degree of segregation and specialization in the spinal and cerebellar projection to the LVN. In their experiments, they identified five microzones in the cerebellar vermis (b zone) that received varying mixtures of convergent input from the fore- and hindlimb afferents and that projected to VSNs that also received direct input via the ventral spino-olivocerebellar pathway, also from the fore- and hindlimbs. Thus the direct inputs from the spinal cord and the indirect ones, via the cerebellum, were somatotopically matched. Such an organization would provide a suitable circuit by which cerebellar activity could shape direct excitatory input. Overall, it is probable that the discharge patterns observed in the VSNs reflect the integration of highly convergent inputs from a number of diverse sources together with a powerful inhibitory sculpting from the cerebellum.

It is also worth emphasizing that, in contrast to the MRF, which receives abundant input from the motor cortex (Kably and Drew 1998a; Keizer and Kuypers 1984; Kuypers 1958; Lamas et al. 1994; Matsuyama and Drew 1997; Rho et al. 1997), the LVN has little cortical input, particularly from area 4 (Akbarian et al. 1993; Licata et al. 1990; Wilson et al. 1999). This implies that the only means by which a descending cortical command for movement may modify VSN activity in the LVN is, indirectly, via the powerful cerebellar projections.

Discharge characteristics of type B and C VSNs

The second most common type of discharge activity was that described by the type B cells that showed only a single distinct trough at the same time as trough 1 of the double-burst cells. Some of these cells also showed a sharp peak of activity just prior to this trough. It is possible that this class of cell may be...
influenced by, and/or influence, both hindlimbs as the sharp pulse of activity that was seen in these cells at the end of the ipsilateral extensor period was time-locked to the onset of the period of activity of the coGL. In addition, given that the period of activity of these type B cells often exceeded the period of activity of the ipsilateral hindlimb extensors and that the discharge started well before the onset of activity of the iVL, it is possible that these cells may exert a bilateral influence on both the ipsilateral and contralateral extensors. As such, it is possible that these type B cells might represent that sub-group of VSNs that receive input only from the hindlimbs (Allen et al. 1972; Andersson and Oscarsson 1978b). At the same time, we cannot discount an influence of these VSNs on the activity of forelimb muscles.

Last, the type C VSNs discharged in a single, clear phasic burst (see e.g., Fig. 5C). The activity patterns in most of these cells were differentiated from the type B cells by the fact that their period of discharge was shorter, and, correspondingly, the pause (trough 1) was longer than that observed in any of the type B VSNs (Fig. 7D). Moreover, in some of these type C neurons, the discharge activity was time-locked to the period of activity of muscles of a single limb. Consequently, these cells may form a subpopulation of VSNs that have less diffuse termination patterns than the type A and B neurons. It is possible that such neurons might receive cerebellar input from a microzone likewise receiving input from a single limb. Although the paper by Abzug et al. (1974) demonstrated that only 50% of the lumbar projecting cells sent collaterals to the cervical cord, the authors also suggested that this value was probably an underestimate due to the limitations of the methodology used. It is thus possible that the relatively small percentage of cells having this pattern of activity might be indicative of the relatively small percentage of cells that innervate only a single spinal enlargement. It is also worth pointing out that most of these neurons were recorded in only a few penetrations. It is, therefore quite possible that more detailed exploration may show a wider variety of patterns of activity of this type of VSN than those found in the present study.

Comparison with reticulospinal neurons

The discharge characteristics of RSNs differ from those of VSNs both with respect to the pattern of discharge and to the overall discharge frequency. With respect to the former point, all of the VSNs that we recorded showed a strong temporal relationship between at least a part of their discharge activity and the activity of one or more EMGs. In the vast majority of cells, this relationship was between the cessation of activity in the iVL and the end of the period of discharge in peak 2. As such, all of these cells would be classified as EMG related using the same bases as we have used previously for the RSNs (see Drew et al. 1986). In contrast, only 41% of the RSNs were classified as EMG related with the others showing modulation of their activity that was not fixed to activity in any one muscle (locomotor related) or not showing any relationship to the rhythmicity of the locomotion at all. It is unlikely that these differences between VSNs and RSNs can be simply attributed to inter-animal differences as the populations of neurons in each brain stem region were recorded in each cat. Moreover, the discharge patterns of these RSNs were very similar to those that we have described previously (Drew et al. 1986).

Overall, these differences in the discharge pattern suggest that VSNs and RSNs play different roles in the regulation of locomotion. By virtue of the fact that discharge activity in different EMG-related RSNs temporally covaried with the activity of flexor and extensor muscles in different limbs, it is possible that such cells may regulate the activity of muscle groups in one limb independently of those in others. Additionally, the fact that many different patterns of activity were seen suggests that RSNs, on one side of the MRF, are able to selectively modulate the level of muscle tone in different limbs. Such diversity and selectivity might provide the flexibility required to produce postural changes in activity in different circumstances and in different contexts. At the same time, a substantial number of RSNs discharged multiple bursts of activity that temporally covaried with activity in more than one limb. As we have suggested before (Drew et al. 1986), this more diffuse characteristic of RSNs might allow the selection of groups of muscles, in different limbs that would act as a synergistic unit. Such neurons might play a role in producing coordinated postural responses during locomotion and/or in modulating interlimb coordination.

In contrast, the more stereotypical discharge patterns of the VSNs suggest that they play a more general role in the control of locomotion. The generally high tonic discharge level in these cells would be consistent with a role for these neurons in regulating the overall level of muscle tone during locomotion, perhaps in concert with those neurons in the MRF that Mori (1987, 1992) has identified as being important in this context. The fact that the discharge frequency of the majority of VSNs is modulated suggests that they play a role in regulating rhythmic activity, although the lack of any fixed relationship to the pattern of extensor muscle activity suggests that there is no simple relationship between the pattern of VSN activity and the pattern of EMG activity. Rather we suggest that VSNs contribute to the activity of the extensor muscles in several limbs and that the exact timing of the cell discharge may be critically influenced by the pattern of interlimb coordination. In this way, it is possible that feedback from the otoliths, from peripheral receptors, or centrally from interneurons signaling information about the CPG, will modulate the activity of the VSNs to ensure that their maximum output occurs at times in the step cycle at which there is the greatest need for antigravity activity.

Finally, the difference in cortical projection patterns that we discussed in a preceding paragraph suggests differences in the contexts under which these two systems are used. We suggest that the vestibulospinal system is a relatively homogenous tonic system that would be activated in all circumstances in which there is a need to support body weight and whose level of activity would be modulated in parallel with the needs for increased or decreased muscle tonus. Much of this control is likely to be exerted via the cerebellum. The reticulospinal system is more heterogenous, fulfilling a number of functions, one of which would be similar to that of the vestibular system. In addition, however, the presence of the powerful corticospinal pathways and the variability of the phasic discharge patterns suggests that parts of this system may play a more specific role in modulating the level in EMG activity in different limbs and, in particular, in coordinating postural and voluntary activity (see Kably and Drew 1998b).
We acknowledge the technical assistance of N. DeSylva, M. Bourdeau, and J. Lavoie. We also thank Drs. A. Smith and S. Rossignol for comments on the manuscript. This work was supported by a grant from the Canadian Medical Research Council. T. Drew was the recipient of a scholarship from the Fonds de Recherche en Santé du Québec. K. Matsuyama received support from the Canadian MRC and from the FRSQ.

REFERENCES


2256 K. MATSUYAMA AND T. DREW

ORLOVSKY GN. Work of the reticulo-spinal neurons during locomotion. Bio-


UDO M, MATSUKAWA K, KAMEI H, MINODA K, AND ODA Y. Simple and complex spike activities of Purkinje cells during locomotion in the cerebel-


UDO M, ODA K, TANAKA K, AND HIROKAWA J. Cerebellar control of locomo-


